PERGAMON

Survival of *Sitophilus oryzae* (L.) on wheat treated with diatomaceous earth: impact of biological and environmental parameters on product efficacy ☆

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Abstract

A series of experiments was conducted to evaluate the effects of temperature, relative humidity (r.h.), population density, concentration, exposure interval, and residual aging on susceptibility of *Sitophilus oryzae* (L.), the rice weevil, to diatomaceous earth (DE). In the first experiment, hard red winter wheat was treated with 300 ppm of the Protect-It™ formulation of DE, and 10, 20, or 30 1–2 week-old mixed-sex adult weevils were exposed on 35 g of wheat for 1 week at combinations of 22°C, 27°C, or 32°C; 40%, 57%, or 75% r.h. No weevils survived when exposed at 40% or 57% r.h., but at 75% r.h. survival was related to both population density and temperature. A higher percentage of adults survived when 30 were exposed compared to 10 and 20, and within each density, survival decreased with increasing temperature. No F_1s were produced at any r.h. on wheat held at 22°C. At 27°C and 32°C, the maximum number of F_1s was produced on wheat held at 75% r.h. In the second experiment, wheat was treated with 25%, 50%, 75%, or 100% of the label rate of 300 ppm, and 10 mixed-sex adult *S. oryzae* were exposed on 35 g of wheat for either 1, 2, or 3 weeks at 27°C, 57% and 75% r.h. Survival decreased with increasing exposure interval and concentration, but within exposure interval and concentration, survival was usually greater at 75% versus 57% r.h. In the final experiment, wheat was treated with 300 ppm, held at 22°C and 27°C, 57% r.h., and bioassayed at monthly intervals for 3 months by exposing 20 adult mixed-sex *S. oryzae* on 35 g of wheat for 1 or 2 weeks. At each month, survival of *S. oryzae* was greater when exposed at 22°C compared to 27°C and when exposed for 1 week compared with 2 weeks. Survival gradually increased with each monthly bioassay, except for those conducted at 3 months. Results of these studies show that *S. oryzae* is susceptible to DE, but survival of exposed insects will depend in part on the temperature and r.h. humidity (or grain moisture content) at which they are exposed. Survival is directly related to temperature, and as r.h. increases either

higher concentrations or longer exposure intervals will be necessary to maintain a certain level of mortality. There may also be a loss of efficacy with residual aging. Published by Elsevier Science Ltd.

1. Introduction

Contact insecticides applied directly to wheat as it is loaded into storage have historically been important components of grain management programs to maintain product quality. These insecticides are primarily organophosphorus and pyrethroid compounds, and the residues from this single application can often prevent insects from becoming established in stored wheat. However, the development of resistance to several important insecticidal grain protectants (Subramanyam and Hagstrum, 1995), an increasing emphasis on testing and evaluating non-toxic controls (Arthur, 1996), and a possible loss of organophosphate grain protectants in the United States due to regulatory action have intensified research efforts for alternative controls that can replace conventional insecticides in stored grains.

Inert dusts, including diatomaceous earth (DE) products, are receiving increased attention for direct application to raw grains. New formulations are more effective than older products, and there are several commercial products manufactured from either freshwater or marine deposits (Quarles and Winn, 1996). Inert dusts disrupt the epicuticle by absorption of lipids, and insects are more vulnerable to desiccation once they lose the protection of the waterproof layers. The insecticidal efficacy of DE varies among products, and can be affected by physical properties of the DE, the temperature and relative humidity (r.h.) at which insects are exposed, and the target insect species (Golob, 1997; Korunic, 1998).

The formulation of the DE of marine origin, Protect-It™, is labeled for use at the rate of 300 ppm for surface treatment on raw grains. Among the insects listed on the label is the rice weevil, *Sitophilus oryzae* (L.), a major internal pest of stored wheat. The female weevil oviposits directly into the kernel, the larva hatches and feeds on the germ, and the adult bores out of the kernel when development is complete. *Sitophilus oryzae* is often difficult to control with insecticides because the larvae are protected inside the kernels. The objectives of this study were to determine: (1) the effect of temperature, r.h., and insect density on survival and F_1 production of *S. oryzae* exposed on wheat treated with DE; (2) the effect of exposure interval and concentration on insect survival; and (3) the impact of storage temperature on residual efficacy.

2. Materials and methods

2.1. Experiment 1: Effect of temperature, r.h., and insect density on survival of S. oryzae and F_1 production

Plastic humidity chambers were created by pouring 750 ml of saturated K_2CO_3, NaBr, or NaCl into three sets of $26 \times 36.5 \times 15\,cm^3$ plastic boxes with waffle-type grids cut to fit the bottom. These solutions maintained humidity at approximately 40%, 57%, and 75%, respectively

(Greenspan, 1977). One complete set of chambers was then put into each of three temperature incubators set at 22°C, 27°C, and 32°C. A HOBO data logger (Onset Computer Corporation, Pocasset, MA, USA) was put in each chamber to monitor temperature and r.h.

The experimental unit for this study consisted of 35 g of hard red winter wheat weighed into a 92 mm tall × 26 mm diameter plastic vial. The Protect-It™ formulation of DE was mixed with wheat at the label rate of 0.3 mg/g of wheat (0.6 lbs/ton, 300 ppm); therefore, the equivalent rate for the 35 g of wheat in the vial was 10.5 mg. At each temperature–humidity combination, densities of either 10, 20, or 30 mixed-sex 1–2 week-old adult *S. oryzae* were exposed for 3 weeks in separate vials containing the treated wheat. Each individual vial was prepared by pouring the wheat from the vial into a beaker, adding the DE, placing the rice weevils into the empty vial, then returning the treated wheat to the vial. There were five treated replicates at each density, plus an untreated control replicate (18 total vials for each temperature–humidity combination). Separate trials were conducted randomly at each temperature–humidity combination.

Weevils were exposed on the treated wheat for 3 weeks, removed from the wheat, and classified as live or dead, then discarded. The wheat was returned to the chambers, and held for 3–4 weeks until F_1 adults emerged. The number of live and dead F_1s were counted weekly for 3 weeks and added together, because all these F_1s had successfully emerged from the kernel. After 3 weeks, the wheat was discarded, and the weekly counts were totaled.

Data for live adults at the different population densities of 10, 20, and 30 were converted to percentage values. The ANOVA procedure of the Statistical Analysis System (SAS, 1987) was used to determine the significance of population density, temperature, and r.h. on weevil survival after 3 weeks of exposure on the treated wheat and the number of F_1 adults. Significance among population densities or temperature within a specific humidity was determined using the General Linear Model (GLM) and the Kruskal–Wallis test under the NPAR1WAY procedure of SAS. There was little mortality in untreated controls, and when corrections for mortality were necessary they were done using Abbott's (1925) formula.

2.2. Experiment 2: Effect of concentration and exposure interval on survival of S. oryzae

Based on the results of the first test, a second test was initiated at 27°C, 57% and 75% r.h. to determine the effect of concentration of DE and exposure interval on weevil survival. At each combination, ten 1–2 week-old mixed-sex adult *S. oryzae* were exposed for either 1, 2, or 3 weeks in vials containing wheat treated with 71, 143, 214, and 300 ppm (2.5, 5, 7.5, and 10.5 mg of DE per 35 g of wheat). There were four treated replicates plus an untreated control replicate for each exposure interval and concentration (60 vials), at each temperature–humidity combination. Experimental methods and procedures for treating the wheat were as described for Experiment 1. Upon completion of the exposure interval, the wheat was sifted, weevils were classified as live or dead, then discarded along with the wheat.

This test was also analyzed using the ANOVA procedure of SAS to determine significance of exposure interval (1, 2, or 3 weeks), r.h., and concentration of DE on weevil survival. Within each temperature and r.h., the Waller–Duncan k-ratio t-test was used to determine the significance of exposure interval on survival at each concentration. Any corrections for mortality in controls were made using Abbott's (1925) formula.

2.3. Experiment 3: Residual efficacy of DE applied to wheat and stored at different temperatures

The results of Experiment 2 indicated that a 1–2 week exposure interval was sufficient to kill *S. oryzae* exposed on wheat treated with the labeled rate of Protect-It™ (300 ppm) and held at 57% r.h. In this test, 9.0 mg of DE was added to 64 individual plastic vials (80 mm tall × 24 mm diameter) containing 30 g of wheat so that the wheat was treated with DE at the rate of 300 ppm, following procedures described in the previous tests. An untreated control with a total of 16 vials was also included in the test. Vials containing the treated wheat and the untreated wheat were held in one of the two plastic boxes containing NaBr to maintain a r.h. of 57%. These plastic boxes were stored inside incubators at either 22°C or 27°C. Bioassays were conducted at month 0 (1 day after treatment), and after vials had been stored for 1, 2, and 3 months, by exposing *S. oryzae* for 1 or 2 weeks in separate vials.

For each residual bioassay, a specific set of vials was removed from the humidity boxes and the incubators. Twenty 1–2 week old mixed-sex *S. oryzae* were put in each individual treated replicate vial and the untreated controls returned to the humidity box in each temperature incubator, and exposed for either 1 or 2 weeks. Upon completion of the exposure interval, mortality was assessed, the beetles were transferred to vials containing untreated wheat, and returned to the humidity chambers. After 1 week of being held on the untreated wheat, mortality was assessed again, and the wheat was discarded. The test was analyzed using the ANOVA procedure of SAS, with temperature, exposure interval, and bioassay month as main effects, and survival after exposure and after 1 week on untreated wheat as a repeated measure. When necessary, corrections for control mortality were made using Abbott's (1925) formula.

3. Results

3.1. Experiment 1: Effect of temperature, r.h., and insect density on survival of S. oryzae and F_1 production

Survival of *S. oryzae* on untreated controls held at 40% r.h. was $100 \pm 0.0\%$, $35.0 \pm 12.6\%$, and $29.0 \pm 0.0\%$ at 22°C, 27°C, and 32°C, respectively, while survival at 57% and 75% r.h. ranged from $95.7 \pm 3.0\%$ to 100% at all three temperatures. No weevils survived when exposed on wheat treated with 300 ppm DE at either 40% or 57% r.h., therefore, survival data were analyzed only for weevils exposed at 75% r.h. Population density ($F = 6.4$, df = 2, 27) and temperature ($F = 31.8$, df = 2, 27) were both significant ($P < 0.01$) but the interaction was not ($P = 0.89$). Survival was related to both density and temperature. A higher percentage of weevils survived when 30 adults were exposed compared to 10 and 20, and within each density, survival was greatest for weevils exposed at 22°C versus 27°C and 32°C (Table 1). Survival at 22°C was $42.5 \pm 11.8\%$, $56.2 \pm 13.9\%$, and $74.0 \pm 3.1\%$ for densities of 10, 20, and 30 weevils, respectively, while survival at 27°C and 32°C did not exceed $25.2 \pm 13\%$.

The number of F_1 adults in each individual control replicate ranged from 56 to 300 at 27°C and from 1 to 207 at 32°C, depending on the density (Table 2). In these treatments, population density ($F = 49.0$, df = 2, 81), temperature ($F = 317.8$, df = 2, 81), r.h. ($F = 481.6$, df = 2, 81), and the r.h. ∗ temperature and the r.h. ∗ density interactions were all highly significant ($P < 0.01$) for F_1

Table 1
Survival (mean% $\pm$ SEM) of 10, 20, or 30 adult *S. oryzae* exposed for 1 week on wheat treated with 300 ppm DE and held at 22°C, 27°C, and 32°C, 75% r.h.[a]

Temperature (°C)	Population density		
	10	20	30
22	42.5 $\pm$ 11.8aB	56.3 $\pm$ 13.9aAB	74.0 $\pm$ 3.1aA
27	7.5 $\pm$ 2.5bB	7.5 $\pm$ 3.2bB	25.3 $\pm$ 13.7bA
32	0.0 $\pm$ 0.0bB	0.0 $\pm$ 0.0bB	20.8 $\pm$ 9.9bA

[a] At each density, means for survival of *S. oryzae* exposed at 22°C, 27°C, and 32°C (columns, lower case letters) and for each temperature, survival at each density (rows, upper case letters) followed by the same letter are not significantly different ($P \geqslant 0.05$, Waller–Duncan k-ratio t-test).

Table 2
Number of F_1 adult *S. oryzae* in the untreated control replicates when 10, 20, and 30 mixed-sex parent adults were exposed for 1 week on wheat held at 27°C and 32°C; 40%, 57%, and 75% r.h.

Density	% r.h.	F_1 adults at 27°C	F_1 adults at 32°C
10	40	56	1
	57	212	38
	75	200	124
20	40	100	100
	57	250	117
	75	300	150
30	40	167	2
	57	275	207
	75	300	200

progeny. No F_1s were produced at 22°C even though more weevils survived compared to exposures at 27°C and 32°C, so only data for these latter two temperatures were analyzed for differences among r.h. with respect to temperature and density. At 27°C, the number of F_1s at each density progressively increased as the r.h. increased (Table 3), with maximum levels in wheat held at 75% r.h. Approximately 200 progeny were produced from the vials in which 20 and 30 adults were exposed at 75% r.h. Fewer progeny were produced at 32°C compared to 27°C, except when 30 parent adults were exposed (Table 3); however, maximum reproduction still occurred in wheat held at 75% r.h.

3.2. Experiment 2: Effect of concentration and exposure interval on survival of S. oryzae

Survival of *S. oryzae* on untreated wheat was 99.2 $\pm$ 0.8% on wheat held at 57% r.h. and 100% on wheat held at 75% r.h. Main effects r.h. ($F = 46.8$, df $= 1$, 72), concentration ($F = 26.0$,

Table 3
Number of F_1 adult S. oryzae (mean$\pm$SEM) produced when 10, 20, and 30 mixed-sex parent adults were exposed for 1 week on wheat treated with 300 ppm DE and held at 27°C and 32°C; 40%, 57%, and 75% r.h.[a]

Density	% r.h.	F_1 adults at 27°C	F_1 adults at 32°C
10	40	1.5$\pm$0.6cA	9.2$\pm$9.2bA
	57	32.0$\pm$1.1bA	4.0$\pm$0.9bB
	75	106.7$\pm$22.0aA	68.7$\pm$13.4aA
20	40	3.7$\pm$1.1cA	0.2$\pm$0.0cA
	57	85.8$\pm$2.5bA	6.5$\pm$0.9bB
	75	190.2$\pm$4.1aA	100.0$\pm$10.0aB
30	40	6.2$\pm$0.7cA	7.5$\pm$7.5bA
	57	83.7$\pm$7.7bA	10.5$\pm$3.7bB
	75	187.5$\pm$12.5aA	200.0$\pm$18.5aA

[a] For each density, means within temperature for the number of F_1 adults at 40%, 57%, and 75% r.h. (columns, lower case letter) and means for number of F_1 adults at 27°C and 32°C for each r.h. (rows, capital letters) followed by the same letter are not significantly different ($P\geqslant0.05$, Waller–Duncan k-ratio t-test).

Table 4
Percentage survival (mean$\pm$SEM) of S. oryzae exposed for 1, 2, and 3 weeks on wheat treated with 71, 143, 214, and 300 ppm DE and held at 57% and 75%[a]

% r.h.	Week	Concentration of DE (ppm)			
		71	143	214	300
57	1	50.0$\pm$4.1a	8.0$\pm$4.1a	5.0$\pm$2.9a	2.5$\pm$2.5a
	2	0.0$\pm$0.0b	0.0$\pm$0.0b	0.0$\pm$0.0a	0.0$\pm$0.0a
	3	0.0$\pm$0.0b	0.0$\pm$0.0b	0.0$\pm$0.0a	0.0$\pm$0.0a
75	1	100.0$\pm$0.0a	72.5$\pm$15.5a	30.0$\pm$14.7a	17.5$\pm$14.4a
	2	30.0$\pm$14.7b	15.5$\pm$6.4b	0.0$\pm$0.0b	0.0$\pm$0.0a
	3	22.5$\pm$8.50b	0.0$\pm$0.0b	0.0$\pm$0.0b	0.0$\pm$0.0a

[a] For each r.h. and concentration, means for survival of S. oryzae exposed for 1, 2, and 3 weeks followed by the same letter are not significantly different ($P\geqslant0.05$, Waller–Duncan k-ratio t-test).

df$= 3$, 72), exposure interval ($F = 60.1$, df$=2$, 72) and all interactions except r.h.$*$concentration$*$exposure interval were significant ($P<0.01$) for the different concentrations of DE. Survival of S. oryzae after 1 week on wheat treated with 71 ppm DE and held at 57% r.h. was 50.0$\pm$4.1%, but did not exceed 8.0$\pm$5.2% at any other concentration (Table 4). No weevils survived after 2 and 3 weeks of exposure. At 75% r.h., survival at each concentration was greatest after 1 week, and as concentration increased, survival decreased at each of the exposure intervals (Table 4). No weevils survived beyond one week when exposed on wheat treated with either 214 or 300 ppm of DE.

Table 5
Survival of *S. oryzae* (mean% $\pm$ SEM) exposed for 1 and 2 weeks at 22°C and 27°C, 0–3 months post-treatment on wheat treated with DE at the rate of 300 ppm[a]

Month	Temperature (°C)	1 week	2 weeks
0	22	15.6 $\pm$ 6.0aA	1.8 $\pm$ 0.9aB
	27	1.9 $\pm$ 1.9bA	0 $\pm$ 0.0aA
1	22	37.5 $\pm$ 5.7aA	18.1 $\pm$ 5.3aB
	27	4.4 $\pm$ 2.0bA	0 $\pm$ 0.0bA
2	22	46.8 $\pm$ 3.2aA	31.2 $\pm$ 6.6aB
	27	0 $\pm$ 0.0bA	0 $\pm$ 0.0bA
3	22	12.8 $\pm$ 4.3aA	0 $\pm$ 0.0aB
	27	0.6 $\pm$ 0.6bA	0 $\pm$ 0.0aA

[a] Means for survival of *S. oryzae* exposed at 22°C compared with 27°C (columns, lower case letters) and survival after exposure for 1 and 2 weeks (rows, capital letters) followed by the same letter are not significantly different ($P \geqslant 0.05$, Waller–Duncan *k*-ratio *t*-test).

3.3. Experiment 3: Residual efficacy of DE applied to wheat and stored at different temperatures

Survival of *S. oryzae* on untreated controls ranged from 93.7 $\pm$ 3.8% to 100% for the different bioassay months. Main effects residual bioassay ($F = 11, 9$, df = 3, 48), exposure interval ($F = 14.0$, df = 1, 48), temperature ($F = 74.0$, df = 1, 48), and the repeated survival after initial exposure versus survival after 1 week ($F = 18.2$, df = 1, 63) were all significant ($P < 0.01$) on the treated wheat. All interactions were significant ($P < 0.01$) except those where residual time period was included ($P \geqslant 0.05$). At each month, more *S. oryzae* survived when exposed on treated wheat at 22°C compared to 27°C, and survival decreased when weevils were exposed for 2 weeks compared to 1 week (Table 5). None of the comparisons for exposure were significant for *S. oryzae* exposed at 27°C ($P \geqslant 0.05$), but all weekly comparisons at 22°C were significant ($P < 0.05$). Survival gradually increased from the 0-month to the 2-month bioassays, but at month 3 survival was greatly reduced. There was no corresponding decrease in survival in untreated controls.

4. Discussion

The results of these studies show that on wheat treated with DE, survival of *S. oryzae* increases with increasing r.h., and this effect was evident both in the initial survival of *S. oryzae* and the number of F_1 adults (Experiment 1). When weevils were exposed for 1 week on wheat treated with half the label rate of DE (150 ppm), survival was 8.0 $\pm$ 5.2% and 72.5 $\pm$ 15.5% at 57% versus 75% r.h. (Experiment 2). The relationship between r.h. or grain moisture and insect survival has been noted and summarized in several recent tests where other stored-product insect species were exposed to various formulations of DE (Golob, 1997; Korunic et al., 1996; Korunic et al., 1998; Fields and Korunic, 2000; Arthur, 2000, 2001).

The increased survival of *S. oryzae* at higher r.h.s could also be related to the interaction of temperature and r.h. on intrinsic growth rates and population development. *S. oryzae* was classified by Howe (1965) as requiring moderate temperatures and high r.h. for optimal growth, and the rate of population increase greatly declines as temperature increases and r.h. decreases (Hardman, 1978; Beckett et al., 1994). Developmental times of life stages may increase and adult survivorship may decrease at combinations of high temperature and low r.h. (Evans, 1982; Hagstrum and Milliken, 1988). In Experiment 1, there was low F_1 production in both the untreated control replicate and the treated replicates at 32°C and 40% r.h., but the humidity effects were less apparent at 27°C in either controls or treatments.

The effects of temperature on the efficacy of DE products can be variable, and may be related to species and exposure conditions. Fields and Korunic (2000) showed both positive and negative impacts on survival of *S. oryzae*, depending on the specific DE formulations. In their tests, where wheat with 14% moisture content was treated with 400 ppm of the Protect-It™ formulation of DE, survival increased from 40% to 59% at 20°C versus 30°C. In this test, with wheat held at 27°C and 32°C, survival and F_1 production decreased on wheat treated with DE and held at 40% and 57% r.h., but there was no difference between the two temperatures at 75% r.h. The environmental effects of temperature and r.h. may be important when either several DE products are tested against one species or when the response of several species is evaluated to establish an order of susceptibility among these species. Similarly, survival of insects exposed during dose-response tests could depend on the number of insects used in the bioassays as well as the specific exposure condition. In this study, density produced an effect on survival when weevils were exposed at 22°C versus 27°C and 32°C; however, no F_1s were produced at 22°C during the duration of the study. The effect of density on survival has not been examined, in great detail, in exposure studies conducted with stored-product insects.

Published results of studies with DE also imply or state that there is a little degradation or loss in efficacy with time (Korunic et al., 1996), but in this test a progressive increase in survival was noted for the first 2 months of the residual study described in Experiment 3. This effect did not continue, possibly because of either an abrupt decline in wheat quality or a difference in the health and vigor of the *S. oryzae* used in the bioassays at 3 months compared to those used at 0, 1, and 2 months. Even so, survival was consistently greater at 22°C versus 27°C, indicating an increase in toxicity with temperature.

The results of these studies indicate that although *S. oryzae* appears to be very susceptible to DE, survival of exposed insects will depend in part on the temperature and r.h. (or grain moisture content) at which they are exposed. As humidity increases, either higher concentrations or longer exposure intervals will be necessary to maintain a certain level of mortality. The residual efficacy of DE may also be affected by changes in internal characteristics or physical properties of grain while it is stored, but results indicate a potential loss of efficacy with residual aging.

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